each allows specific detection of cells expressing the gene with the retroviral insertion. Moreover, the vectors specifically mark cells expressing the mutant gene, allow temporal and spatial analysis of the phenotype of the disrupted gene, provide for conditional tissue-specific gene inactivation, facilitate the conditional ablation of cell lineages expressing the mutant gene, and facilitate conditional ectopic expression of any gene in any desired tissue. Other important attributes of these vectors include the ability to generate animals with conditional tumors of any cell origin as well as the ability to establish conditional immortal cell lines of any cell type.

Figure 1 depicts the MoMLV-based retroviral vector and its various features. The orientation of the transcriptional units is indicated by arrows. Vectors of other retroviral origin are quite similar to the MoMLV-based vector, differing only in the sequences of the retroviral backbone. The origin and importance of the elements are as follows.

(a) Retroviral Sequences. The retroviral sequences are necessary for packaging and random integration of the incoming DNA into the host genome. The MoMLV sequences are substantially similar to the sequences found in the vector pGen$^-$ (Soriano et al., J. Virol. **65**:2314-2319 (1991)), a vector which lacks the viral enhancer sequences and which contains the bacterial supF gene positioned in the 3' long terminal repeats (LTR). Upon integration into the genome, the 5'LTR enhancer sequences are also deleted, and the supF sequences are copied to the 5'LTR. As described below, the viral LTRs of the parental vector are modified to contain loxP sequences. In addition, the trancriptional orientation of all non-retroviral vector sequences are inverted relative to the transcriptional orientation of the 5' LTR promoter (Fig. 1). Production of high titer stocks from this vector are accomplished following published procedures (for example, Soneoka et al., Nucleic Acids Res. **23**:628-633 (1995); Yee et al., Proc. Natl. Acad. Sci. USA **91**:9564-9568 (1994); and Mann et al., Cell **33**:153-159 (1983)). Alternative retroviral sequences may, for example, be derived from or based upon any lentiviral or ALV vector, and appropriate standard techniques may be used for viral propagation.

(b) LoxP. The loxP sequence is the recognition sequence of the bacteriophage P1 CRE recombinase, and its use is described in Sauer, Meth. Enzymol. **225**:890 (1993). This sequence mediates recombinational excision of the retroviral insertion in the presence of CRE. It also facilitates targeted chromosomal rearrangements, such as translocations and deletions (Ramirez-Solis et al., Nature **378**:720-724 (1995)) in cells containing more than one provirus. Such cells may be obtained through mating of mice, each carrying a different loxP-tagged retroviral insertion. Alternatively, FRT, the recognition sequence of the Saccharomyces cerevisiae FLP recombinase (Dymecki, Proc. Natl. Acad. Sci. **93**:6191-6196 (1996)) may be used for this purpose, and recombinational excision may be mediated by the FLP protein.

(c) V. V, or VDErs, is the recognition sequence of the VDE DNA endonuclease from *Saccharomyces cerevisiae* (Bremer et al., Nucleic Acids Res. **20**:5484 (1992)). This sequence provides a unique chromosomal marker. Other chromosomal markers may also be utilized for this purpose.

(d) Splice Acceptor. As shown in Figure 1, a consensus splice acceptor sequence is also included in the retroviral vectors. This sequence is required for fusion of the retroviral transcripts to the endogenous gene transcript in situations where the retroviral integration occurs in an intron. The splice acceptor site prevents the retroviral transcript from being inadvertently spliced out of the genome, thereby maximizing the likelihood that an insertion is mutagenic for the endogenous gene (Gossler et al., Science **244**:463-465 (1989); Friedrich and Soriano, Genes Dev. **5**:1513-1523 (1991); Skarnes et al., Genes Dev. **6**:903-918 (1992); Takeuchi et al., Genes Dev. **9**:1211-1222 (1995); Wurst et al., Genetics **139**:889-899 (1995); Forrester et al., Proc. Natl. Acad. Sci. USA **93**:1677-1682 (1996); and Brenner et al., Proc. Natl. Acad. Sci. USA **86**:5517-5521 (1989)). A preferable consensus splice acceptor is derived from the Adenovirus major late transcript (Robberson et al., Mol. Cell. Biol. **10**:84-94 (1990)), but any other splice acceptor sequence may be utilized in the vectors of the invention.

(e) Stop Codons. Nonsense codons in all three reading frames ensure translational termination in the gene with the retroviral insertion. Any nonsense codon or set thereof may be used for this purpose.

(f) IRES. The internal ribosome entry site provides for translation initiation of the *tag* gene (described below). As shown, a preferred IRES is derived from the Encephalomyocarditis virus (Morgan et al., Nucleic Acids Res. **20**:1293-1299 (1992)). Other appropriate ribosome entry sites may also be used in the present vectors.

(g) rtTA. The sequence indicated as "rtTA" in Figure 1 is preferably a hybrid protein composed of a mutant tetracycline repressor and the VP16 transcription activation domain (Gossen et al., Science **268**:1766-1769 (1995)). rtTA possesses the ability to stimulate expression of genes placed under the control of the tetracycline operator in the presence of tetracycline derivatives (Gossen et al., Science **268**:1766-1769 (1995)). In the present invention, rtTA is expressed under the control of the promoter of the endogenous cellular gene which has been mutated by the retroviral insertion (Figures 3 and 4). Conditionally expressed rtTA is a key component to functional characterization of genes facilitated by the MAGEKO approach.

(h) pA. As shown in Figure 1, the vectors of the invention also include a polyA addition signal. This signal is required for the processing and expression of the rtTA mRNA. One preferred pA sequence is derived from the bovine growth hormone gene (Goodwin and Rottman, J. Biol. Chem. **267**:16330-16334 (1992)), although any other polyadenylation signal may be used. Examples of other useful pA sequences include, without limitation, the insulin and SV40 pA sequences.

(i) P. P is the constitutively expressed mouse phosphoglycerate kinase-1 (PGK) promoter (Adra et al., Gene **60**:65-74 (1987)). This promoter is required for the expression of GFO (described below). Other constitutive mammalian promoters may be used in place of the PGK sequence.

(j) ATL. ATL, or the adenovirus tripartite leader sequence (Sheay et al.,